In vivo imaging platform for tracking immunotherapeutic cells

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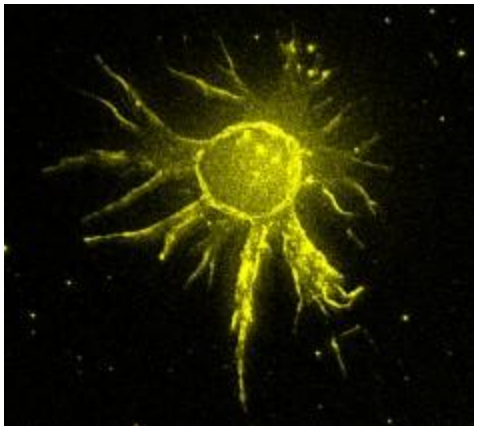
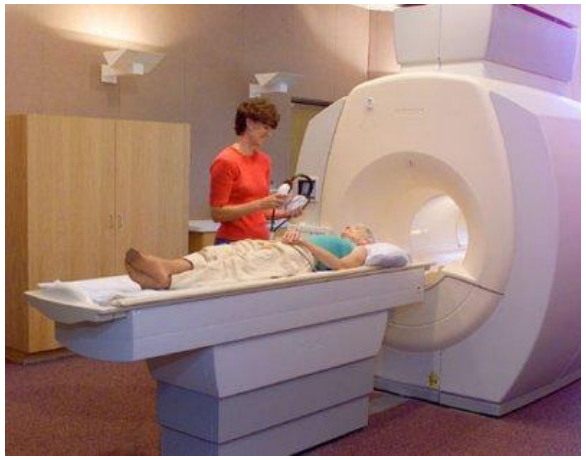
Cellular Therapeutics

- Advantages for cells as therapeutic systems:
  - Ability to carry out complex functions
  - Responsive to changes in surrounding tissues of host organism
- Few noninvasive techniques exist for monitoring cells after administration
- Current monitoring conducted by histological analyses
  - Animal sacrifice
  - Tissue biopsies



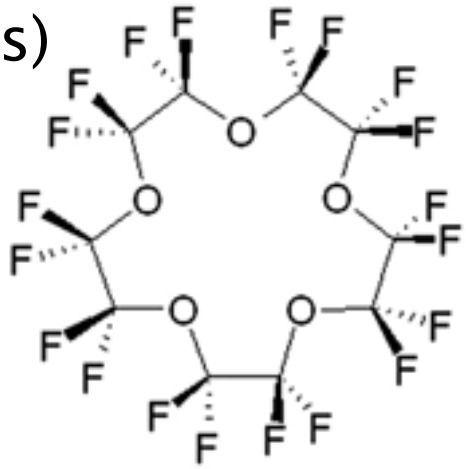
Use of MRI to visualize cells

- Cells incubated with metal-ion based ^{1}H contrast agents *ex vivo* to promote uptake before administration
- Difficult to interpret subtle changes in image contrast of regions with labeled cells
- Large ^{1}H background signal from mobile water makes it difficult to identify transplanted cells *in vivo*



MRI tracking of dendritic cells

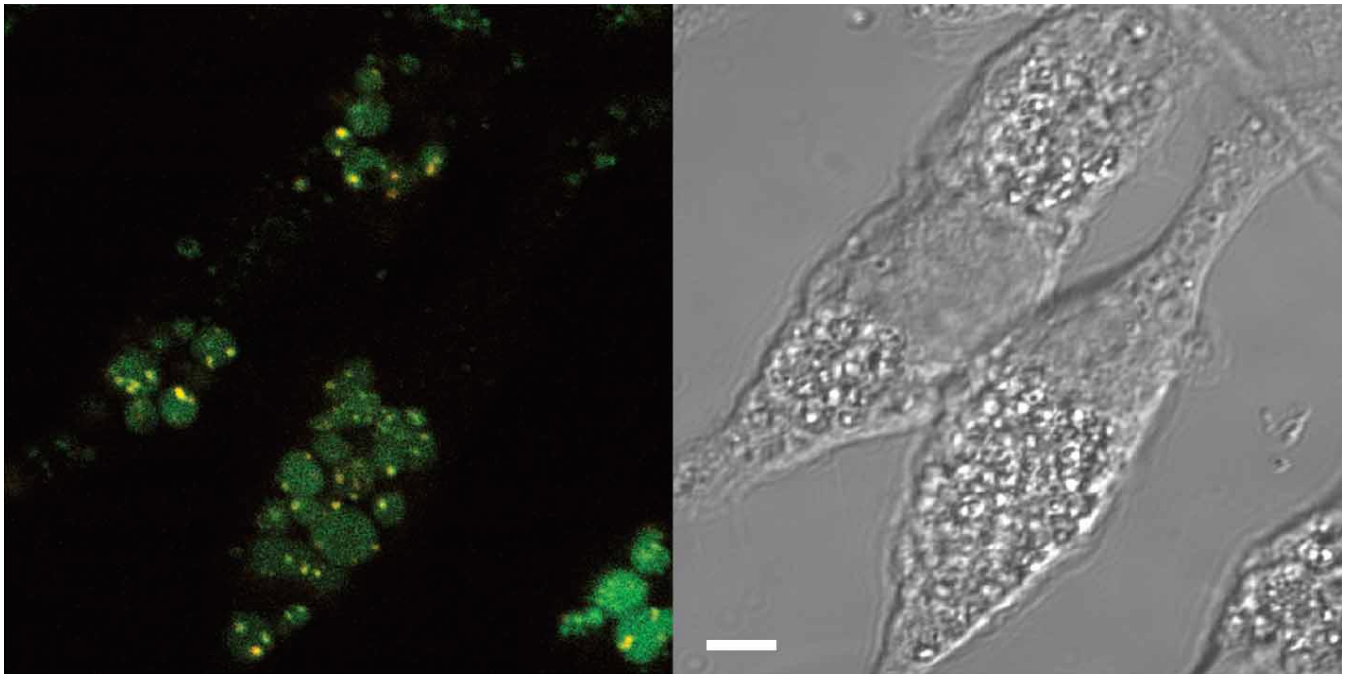
- Cells labeled *ex vivo* with a perfluoropolyether (PFPE) tracer agent
- Administrated to subject and tracked using ^{19}F MRI
- Therapeutic DCs currently evaluated for treatment of various diseases, so they are used for the focus of this study
  - Primary cells from bone marrow (BMDCs)
  - Fetal skin-derived DC line (FSDCs)



PFPE labeling characteristics

- Examined NMR line width, labeling efficiency, and PFPE cellular retention time
  - Single resonance line of ~ 150 Hz, narrow enough for MRI applications
  - Each DC contained on average 0.25 ng of PFPE
  - Intracellular ^{19}F signal ~ 15% of initial value after 5 days

Intracellular incorporation of PFPE



- Antigen retention compartments of FSDCs labeled with FITC-DX
- PFPE particles labeled with cyanine DiI fluorophore with lipofectamine
- Confocal optical section through fixed FSDCs shows colocalization

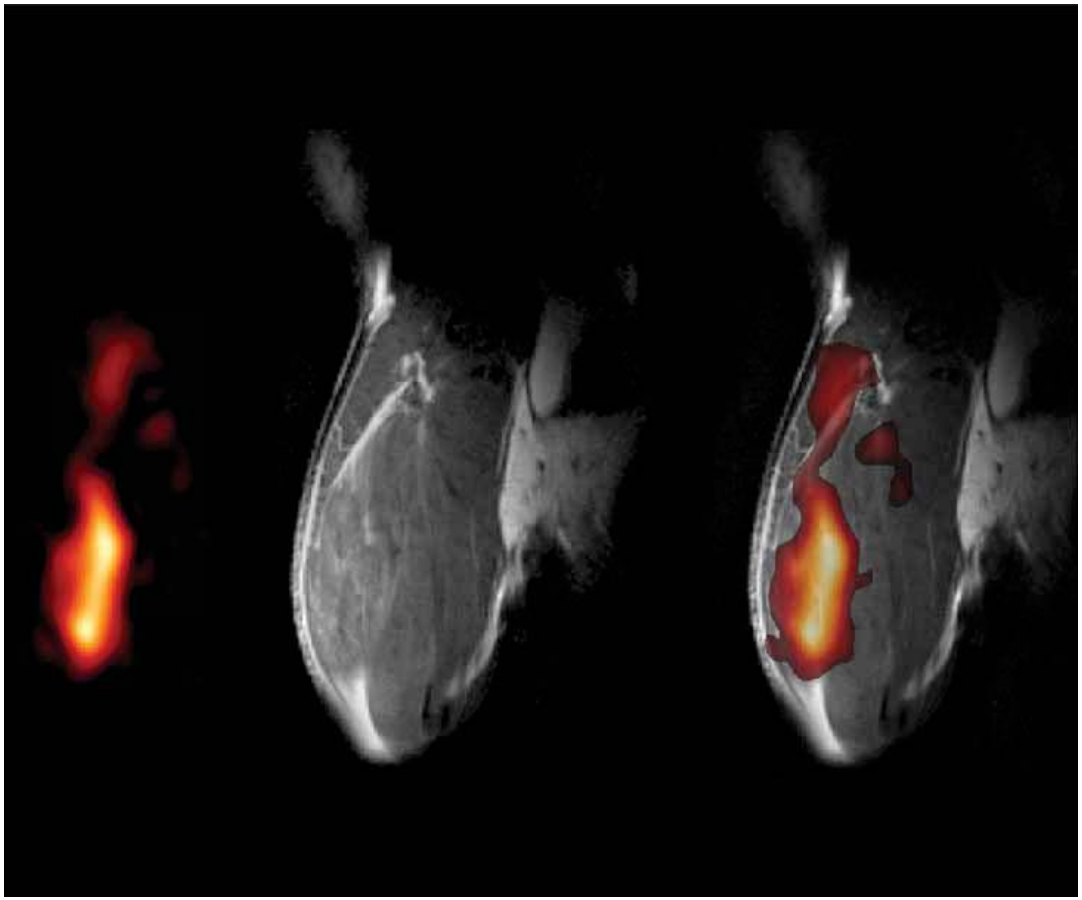
In vitro assays on labeled DCs

- Examined cellular toxicity, proliferation, metabolism, and phenotype
- Cytotoxicity measured by leakage of enzyme glucose 6-phosphate dehydrogenase from cytoplasm into culture medium
  - Little or no apparent cytotoxicity
- Proliferation measured by two methods
  - Methylthiazole tetrazolium (MTT) assay measuring mitochondrial activity
  - Assay of total double-stranded DNA
  - Little or no apparent difference between labeled cells and controls
- No altered phenotype based on flow cytometric analysis of CD80 and MHC class II (maturation markers)

Potential of ^{19}F MRI *in vivo*

- Visualized labeled DCs administered to mice by several routes
  - Focal transplantation directly into tissues
  - Intravenous injection

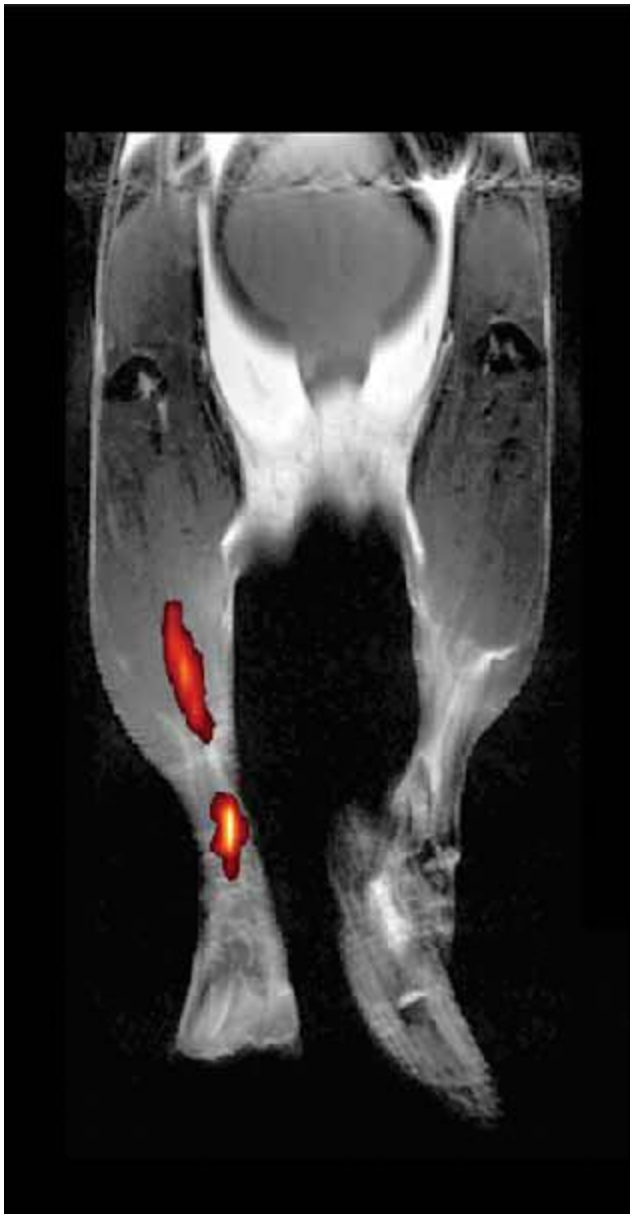
Intramuscular injection



- MRI images of mouse quadriceps 8 h after intramuscular injection of 8 X 10^6 FSDCs
- Middle: T_2-weighted ^{1}H image in same slice plane →
- Right: composite overly image of ^{19}F/^{1}H shows DCs coincident with hyperintensity

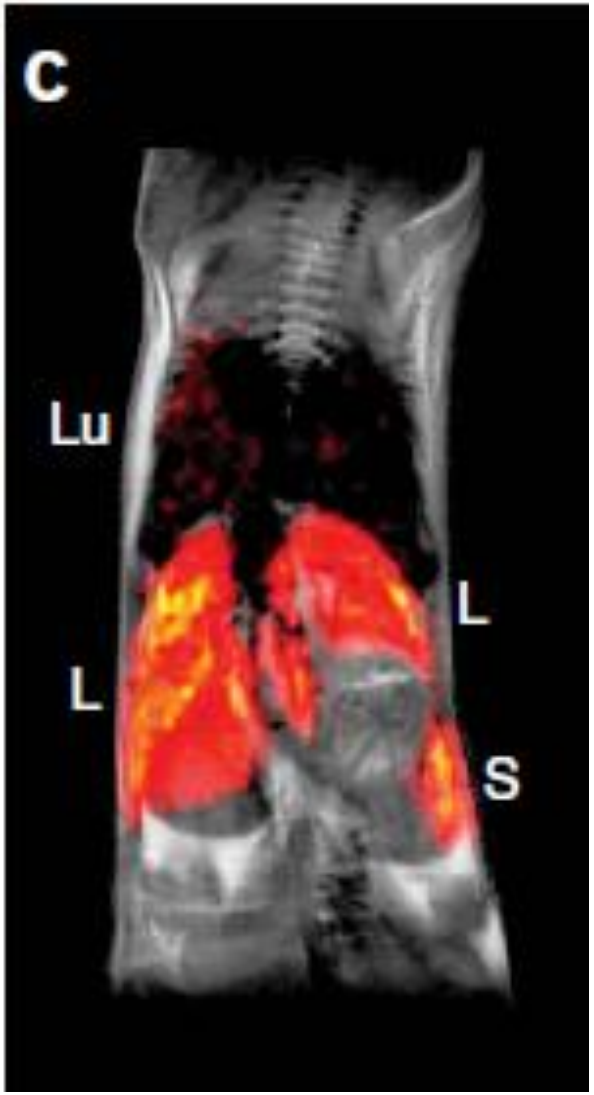
Ability of PFPE-labeled DCs to migrate *in vivo*

- Inject 4 x 10^6 labeled BMDCs subcutaneously into the tip of the hind foot pad
- $^{19}F/^{1}H$ composite 6h post-injection shows cells migrating and accumulating in lymph node
- SNR of cells in node > 10



Intravenous Delivery of PFPE-labeled DCs

- 18 x 10^6 labeled FSDCs injected via the tail vein
- $^{19}F/^{1}H$ composite slice through torso shows pronounced signal in liver and spleen and weakly present in the lungs
- SNR ~ 9



PFPE is a suitable MRI tracer

- ^{19}F spectrum exhibits single narrow resonance
- Large number of NMR-equivalent fluorine atoms per molecule yields high sensitivity
- Minimum number of detectable ^{19}F spins per voxel on same order of magnitude as that of conventional ^{1}H MRI

Issues with PFPE

- False negatives likely because cells must accumulate in sufficient quantities to reach detection threshold
- Can be passed to other cells if primary labeled cell dies and is endocytosed

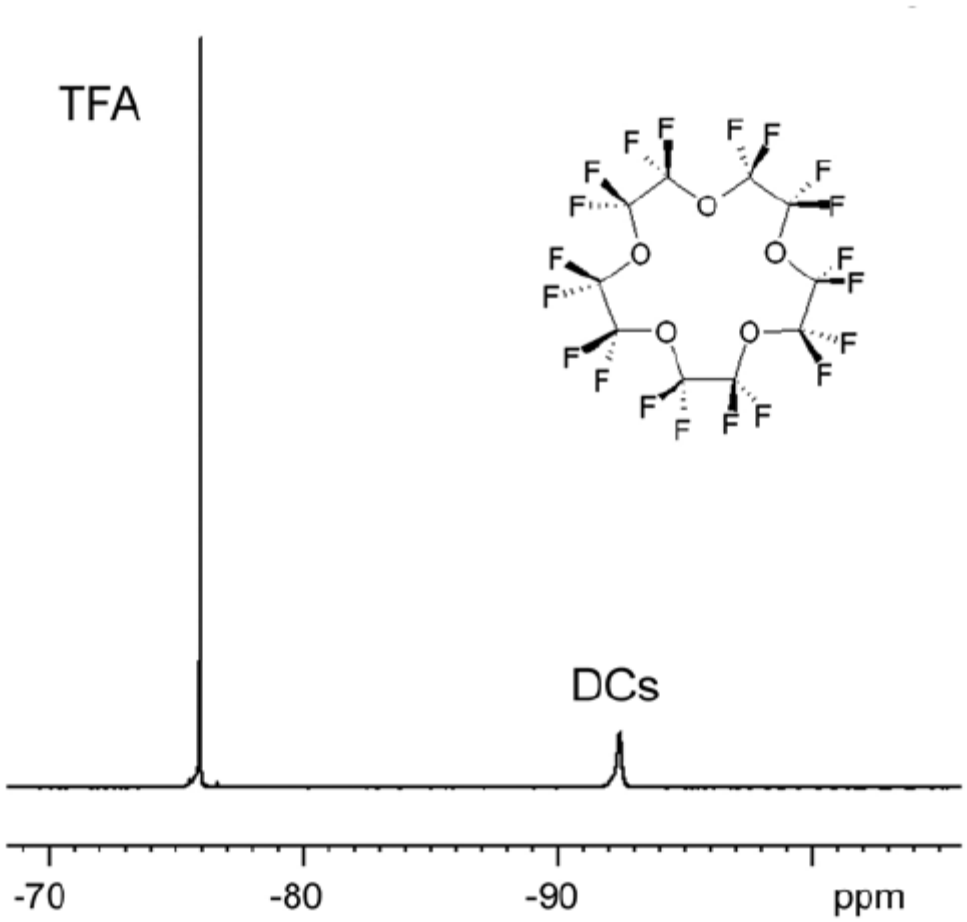
Importance of PFPE-labeled DCs

- Labeling cells before implantation may provide useful way of viewing cell migration for cellular therapeutics
- Technology broadly applicable to different cell types, not just DCs

Questions?

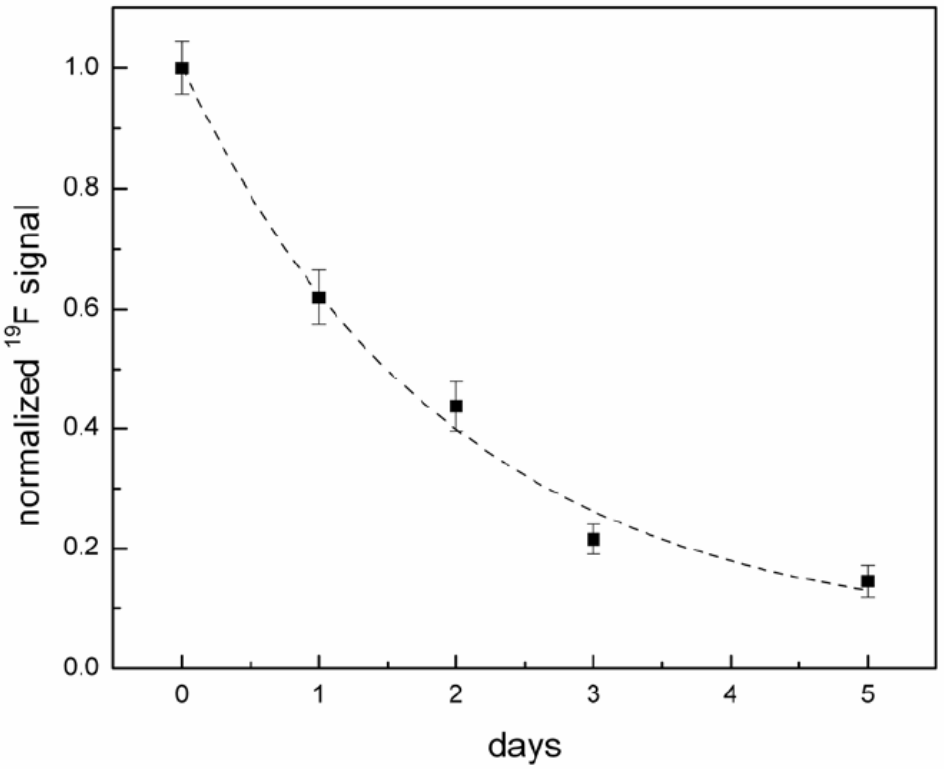
NMR line width

- ^{19}F NMR spectrum of intracellular PFPE emulsion particles in FSDCs (right peak)
- ^{19}F reference compound, TFA, in adjacent capillary tube (left peak)

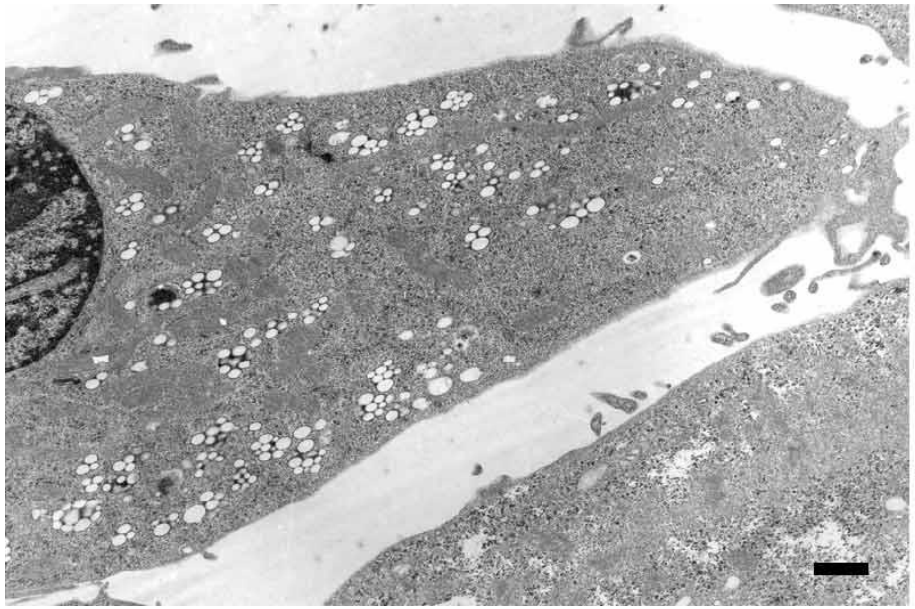


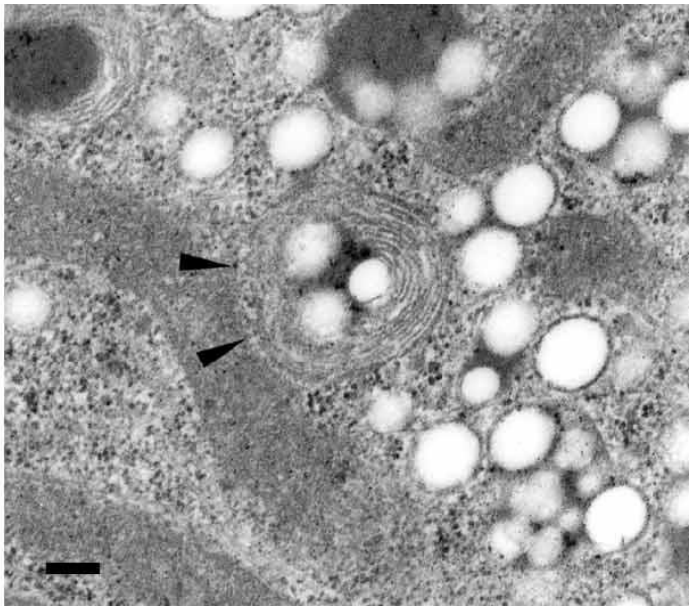
Intracellular retention of PFPE

- Used ^{19}F NMR to monitor labeled FSDCs for 5 days
- ^{19}F signal per cell decreased over time owing to cell division and subsequent dilution of PFPE
- After 5 days, intracellular ^{19}F signal was ~15% of original
- No change in ^{19}F NMR line shape as a function of time, so there is no breakdown of the PFPE molecules



Intracellular incorporation of PFPE with TEM

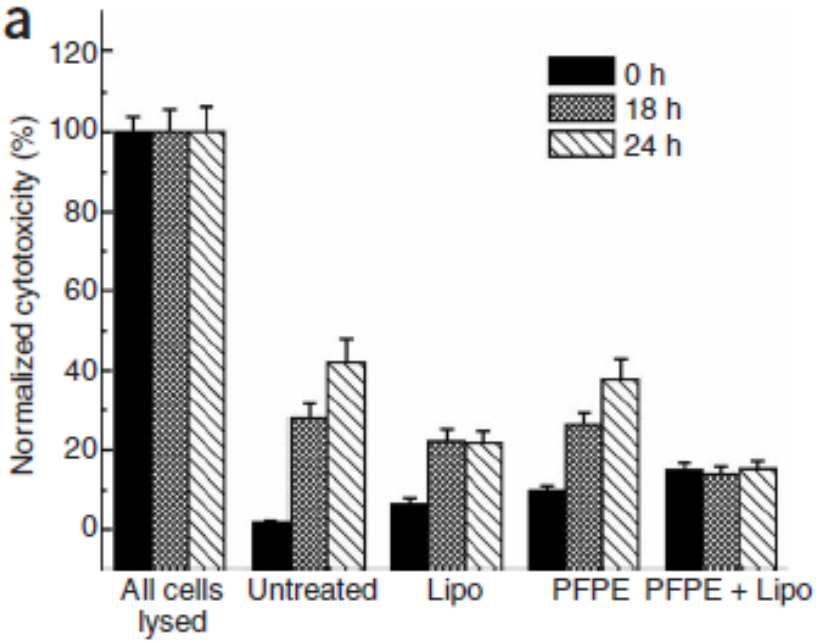




- Intracellular PFPE particles appear as bright smooth spheroids
- Paricles comparmentalized in regions consistent with vacuoles
- Osmium staining used to visualize structures highlighting unsaturated lipids in particles surface
- Small number of particles clustered together surrounded by tightly wrapped multiple membrane compartments, reminiscent of MHCII compartments

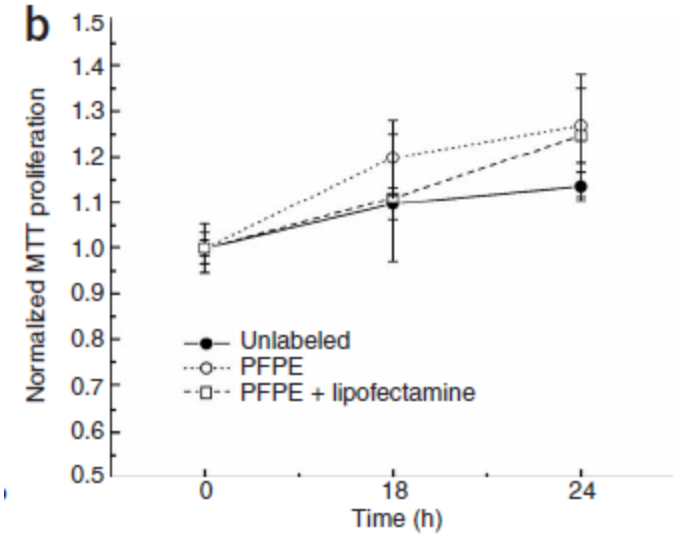
Cytotoxicity of PFPE

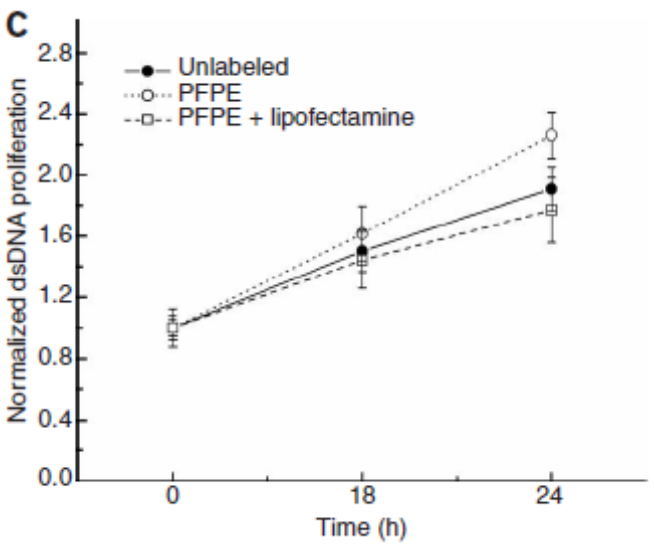
- Measures leakage of the enzyme glucose 6-phosphate dehydrogenase from cytoplasm into culture medium
- Little or no apparent cytotoxicity for all conditions studied
- Right after labeling, some apparent toxicity, particularly when lipofectamine was used
- Cells recover at later time points (18 and 24 hours) and apparent toxicity is no greater than that of the controls



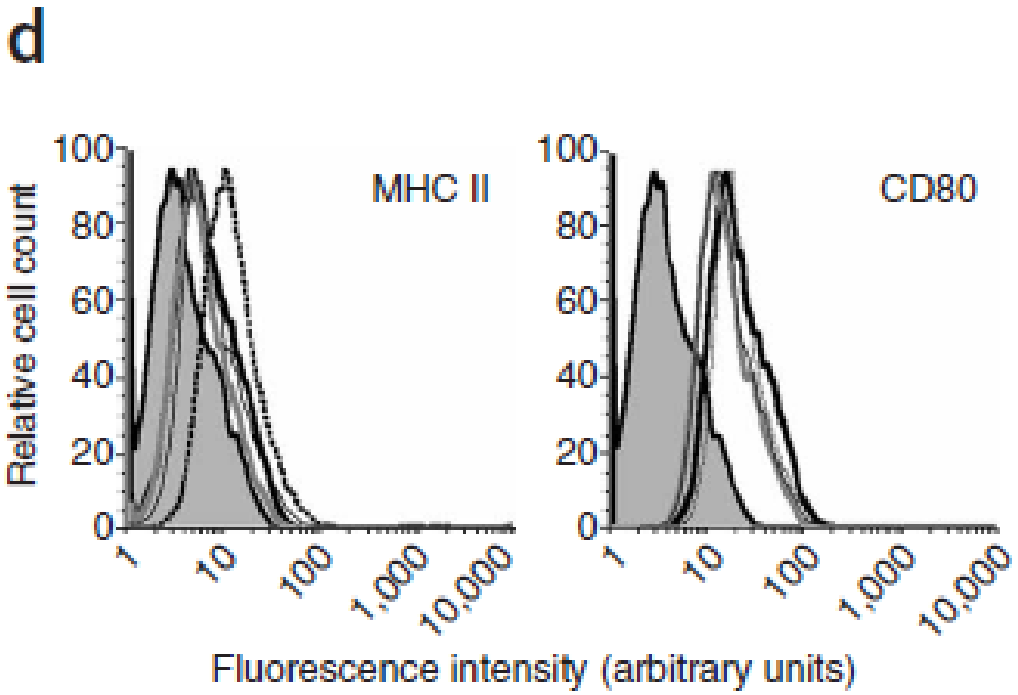
Proliferation assays

- MTT measures mitochondrial activity
- dsDNA assay measures total amount
- Little or no apparent difference in cell proliferation between labeled cells and controls
- Result confirmed by direct cell counts





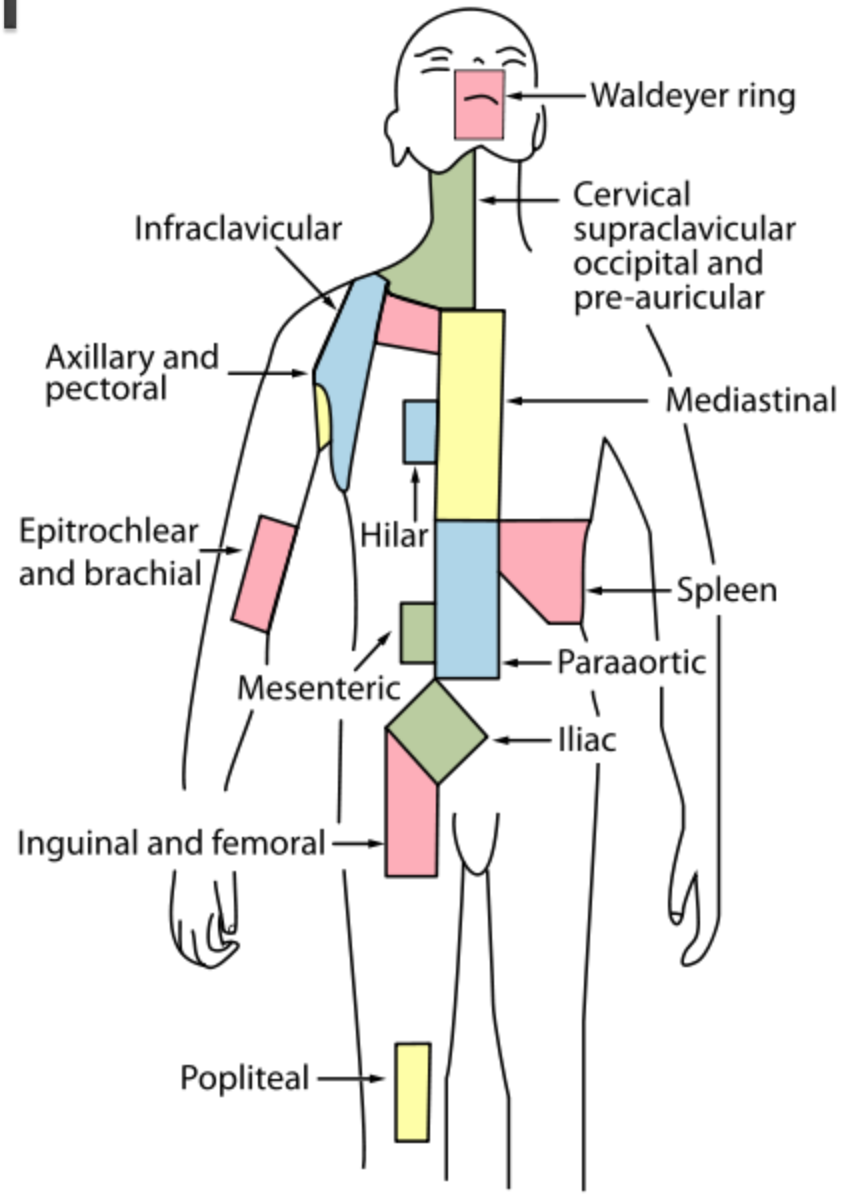
Altered phenotype



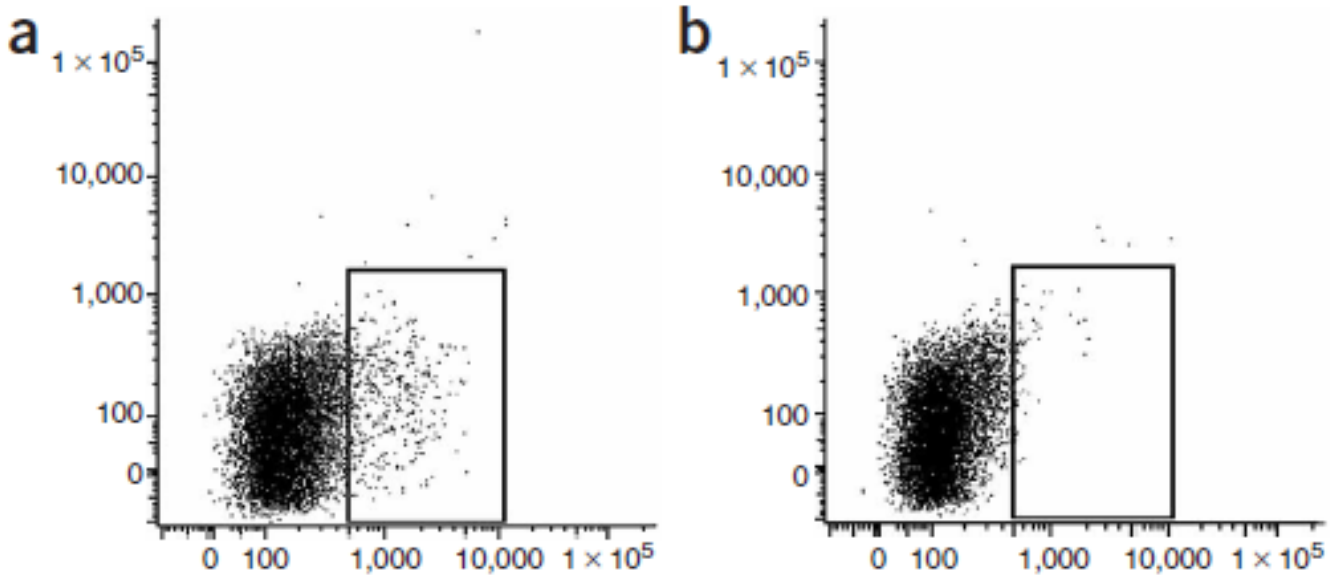
- Flow cytometric analysis of cell surface markers including CD80 and MHC class II
- Any increase in expression levels indicate maturation of DCs, which may alter migratory ability

Migration pattern

- Cells double-labeled with PFPE and 5-chloromethylfluorescein diacetate (CMFDA) and injected them into the foot pad
- 24h post-injection, excised popliteal node as well as inguinal and axillary nodes as controls



FACS analysis of DCs in excised lymph nodes



- A – Fluorescent cells visible in popliteal LNs of mice receiving double labeled DCs
- B – None seen in popliteal nodes when DCs labeled only with PFPE